A Prospective Study of Meat and Meat Mutagens and Prostate Cancer Risk

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Abstract

High-temperature cooked meat contains heterocyclic amines, including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (BaP). In rodents, a high intake of PhIP induces prostate tumors. We prospectively investigated the association between meat and meat mutagens, specifically PhIP, and prostate cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Diet was assessed using a 137-item food frequency questionnaire and a detailed meat-cooking questionnaire linked to a database for BaP and the heterocyclic amines 2-amino-3,8-dimethylimidazo[4,5-*b*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), and PhIP. During follow-up, we ascertained a total of 1,338 prostate cancer cases among 29,361 men; of these, 868 were incident cases (diagnosed after the first year of follow-up) and 520 were advanced cases (stage III or IV or a Gleason score of ≥7). Total, red, or white meat intake was not associated with prostate cancer risk. More than 10 g/d of very well done meat, compared with no consumption, was associated with a 1.4-fold increased risk of prostate cancer [95% confidence interval (95% CI), 1.05-1.92] and a 1.7-fold increased risk (95% CI, 1.19-2.40) of incident disease. Although there was no association with MeIQx and DiMeIQx, the highest quintile of PhIP was associated with a 1.2-fold increased risk of prostate cancer (95% CI, 1.01-1.48) and a 1.3-fold increased risk of incident disease (95% CI, 1.01-1.61). In conclusion, very well done meat was positively associated with prostate cancer risk. In addition, this study lends epidemiologic support to the animal studies, which have implicated PhIP as a prostate carcinogen. (Cancer Res 2005; 65(24): 11779-84)

Introduction

Studies of twins show that up to 50% of prostate cancer cases may be explained by environmental factors, such as diet (1). Meat and fat have generated interest as potential risk factors for this disease, although results from epidemiologic studies have been inconsistent (2). Meat cooked at high temperatures is a source of carcinogenic heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH). The formation of HCAs and PAHs depends on the meat type and is highest in meats cooked by high-temperature cooking methods, such as barbecuing, and in well-done meats (3–6).

One of the most abundant HCAs in cooked meat is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). PhIP increases mutation frequency in the rat prostate (7) and induces prostate tumors in rodent models (8), whereas other HCAs do not (9). Several enzymes required for the metabolic activation of HCAs, including cytochrome P450 1A2 (CYP1A2), CYP1B1, *N*-acetyltransferase 1 (NAT1), and NAT2, are expressed in human prostate tissue (10–12). Furthermore, CYP1A2 is inducible by dietary components, including diets rich in HCAs (13). The data regarding PhIP and prostate cancer risk in humans are limited to one case-control study of 317 cases, which found no association (14).

The aims of this study were to determine whether meat intake or meat-related mutagens, particularly PhIP, was associated with increased prostate cancer risk.

Materials and Methods

Study population. This study was conducted in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a randomized controlled, multisite study (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC) to evaluate selected methods for the early detection of these four cancers (15). Biological samples and questionnaire data were collected to study markers of early detection and etiology of cancer (16).

Between 1993 and 2001, 38,352 men (ages 55-74 years) were randomized to the screening arm of the Trial. At entry, men receive a prostate-specific antigen (PSA) test and digital rectal exam (DRE) for prostate cancer screening and then both PSA and DRE annually for the subsequent 5 and 3 years, respectively. Men with a PSA test result of >4 ng/mL or DRE exam suspicious for prostate cancer are referred to their medical care providers for follow-up. All participants are asked to complete annual questionnaires regarding cancer diagnoses during the previous year.

Participants were ineligible for this study of meat and meat mutagens if they had a history of cancer (other than nonmelanoma skin cancer; n = 1,006); did not have prostate cancer screening results (n = 2,530); lacked any of the following three questionnaires: annual follow-up (n = 1,046), baseline risk factor (n = 250), or food frequency questionnaire (FFQ; n = 6,604); missed more than seven items on their FFQ (n = 250); or were extreme outliers for reported energy intake (those in the top or bottom 1% of intake, n = 634). After exclusions (some individuals for multiple reasons), the cohort consisted of 29,361 men. As the PLCO Cancer Screening Trial is an ongoing randomized clinical trial continuing through 2015, data regarding person-years are not presented in this article.

The study was approved by the institutional review board of the U.S. National Cancer Institute and the Trial screening centers. Written informed consent was obtained from each study participant.

Identification of prostate cancer cases. The PLCO Trial obtains medical/pathologic records for all reported cancer cases and death certificates for all deceased individuals. All prostate cancer cases were

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pathologically confirmed. Incident prostate cancer cases were defined as those diagnosed after the first year of follow-up; therefore, for incident-based analyses, cancers diagnosed within the first year of follow-up were excluded. Tumors classified as stage III or IV according to the tumor-node-metastasis staging of disease classification (ref. 17; based on clinical and, when carried out, surgical findings) or tumors assigned a Gleason score of ≥7 (from either biopsy or prostatectomy, whichever gave the highest value) were considered to be advanced prostate cancer cases. Analyses of advanced prostate cancer included all individuals diagnosed with advanced disease from baseline to the end of follow-up.

Assessment of diet and lifestyle. Upon entry to the study, participants completed a general risk factor questionnaire and a self-administered FFQ (http://www3.cancer.gov/prevention/plco), which asked about the frequency of consumption and portion size of 137 food items during the previous year. Sex- and age-specific portion size and nutrient values were quantified (18). The FFQ included detailed information about meat-cooking methods (barbequing, grilling, pan frying, and broiling) and doneness level (rare, medium, well done, or very well done) for meats commonly cooked by different methods to varying degrees of doneness (steak, bacon, sausage, pork chop, and hamburgers). We used a specifically developed database (http://charred.cancer.gov/) to estimate daily intake of meat mutagens, including the following HCAs: 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and PhIP and the PAH benzo(a)pyrene (BaP; refs. 4–6, 19). This database also enabled the determination of overall mutagenic activity in meat, with values determined by the standard plate incorporation assay with *Salmonella typhimurium* strain TA98, measured as revertant colonies (20).

Statistical analysis. Cox proportional hazards regression, with age as the underlying time metric, was used to estimate relative risks (RR) and 95% confidence intervals (95% CI) for prostate cancer. Relative risks are reported within quintiles, using the first quintile as the referent category. Tests for

Table 1. Mean values for selected characteristics by quintiles of red meat intake

Characteristic	Quintile of red meat intake				
	1	2	3	4	5
Participants (*n*)	5,871	5,873	5,873	5,872	5,872
Cases (*n*)	308	263	275	271	221
Age (y)	64.3	63.8	63.4	62.9	62.1
Family history of prostate cancer (%)	6.9	7.7	7.7	6.8	7.5
Race (%)					
Non-Hispanic White	85.2	90.4	91.6	92.9	93.3
Black	4.4	3.4	3.4	2.8	2.6
Asian/Pacific Islander	8.6	4.2	3.2	2.3	1.8
Hispanic	1.8	2.0	1.7	1.9	2.2
Current body mass index, kg/m^2 (%)					
<25	37.8	28.5	24.6	21.1	17.6
25 to <30	47.4	52.4	51.7	51.1	47.9
≥30	13.2	17.6	22.5	26.3	33.3
Smoking status (%)					
Never	34.7	32.1	29.2	27.5	24.2
Current	6.0	8.2	11.1	12.2	16.3
Former	51.6	51.3	51.9	52.5	51.2
Pipe/cigar only	7.7	8.4	7.8	7.8	8.4
Vigorous physical activity, h/wk (%)					
None	11.3	13.4	14.5	16.3	20.7
0.5	13.2	17.5	18.2	19.7	18.8
1	10.6	11.7	11.8	11.6	11.5
2	15.2	14.9	17.0	15.4	14.6
3	17.0	15.9	14.2	13.8	11.6
≥4	32.7	26.7	24.3	23.2	22.9
Aspirin use (%)					
Nonuse	46.8	49.4	47.8	49.2	47.3
Moderate use (<1/d)	19.9	20.5	22.0	21.8	22.6
History of diabetes (%)	7.0	7.9	8.3	8.9	10.2
Lycopene (mg/d)	9.0	9.8	10.9	12.3	15.5
Supplemental vitamin E (%)					
Nonuse	32.9	38.3	40.8	42.1	45.5
>0 to <30	16.6	17.9	18.6	17.3	16.2
30 to <400	13.9	12.3	10.8	11.4	10.8
≥400	21.0	16.5	14.9	14.6	13.5
Past use	15.6	15.1	14.8	14.6	14.1
Fruits (servings/d)	4.3	3.7	3.3	2.9	2.1
Vegetables (servings/d)	5.8	5.5	5.4	5.4	5.2
Total energy intake (kcal/d)	1,799	1,985	2,221	2,533	3,165

NOTE: All values (except age) are adjusted for age.

Table 2. Distribution and RR with 95% CIs for prostate cancer risk according to quintiles of meat and meat-cooking variables

Variable	Quintile					P_{trend}*
	1 (reference)	2	3	4	5	
Red meat						
Range (g/d)	0.0-43.5	>43.5-68.6	>68.6-98.3	>98.3-146.0	>146.0-845.4	
Total cases (*n*)	308	263	275	271	221	
RR (95% CI) †	1.0	0.89 (0.75-1.05)	0.97 (0.81-1.14)	0.99 (0.83-1.19)	0.91 (0.73-1.12)	0.70
RR (95% CI) ‡	1.0	0.85 (0.70-1.05)	0.94 (0.77-1.16)	1.02 (0.82-1.27)	0.81 (0.62-1.06)	0.38
RR (95% CI)§	1.0	0.82 (0.63-1.08)	0.93 (0.71-1.21)	0.92 (0.69-1.23)	0.92 (0.66-1.29)	0.92
White meat						
Range (g/d)	0.0-22.3	>22.3-36.0	>36.0-53.3	>53.3-84.0	>84.0-873.7	
Total cases (*n*)	249	288	279	270	252	
RR (95% CI) †	1.0	1.15 (0.97-1.36)	1.12 (0.94-1.33)	1.10 (0.92-1.31)	1.06 (0.88-1.29)	0.97
RR (95% CI) ‡	1.0	1.18 (0.95-1.47)	1.28 (1.03-1.59)	1.15 (0.92-1.45)	1.17 (0.92-1.50)	0.52
RR (95% CI)§	1.0	0.98 (0.75-1.30)	1.17 (0.89-1.53)	1.04 (0.78-1.38)	1.01 (0.74-1.37)	0.99
Processed meat						
Range (g/d)	0.0-6.7	>6.7-12.7	>12.7-21.3	>21.3-36.8	>36.8-367.1	
Total cases (*n*)	273	283	260	257	265	
RR (95% CI) †	1.0	1.10 (0.92-1.30)	1.03 (0.86-1.23)	1.03 (0.86-1.25)	1.14 (0.93-1.39)	0.32
RR (95% CI) ‡	1.0	1.14 (0.92-1.41)	1.10 (0.88-1.37)	1.15 (0.91-1.45)	1.16 (0.91-1.50)	0.39
RR (95% CI)§	1.0	1.26 (0.95-1.66)	1.44 (1.08-1.92)	1.07 (0.78-1.47)	1.37 (0.99-1.90)	0.32
Barbecued meat						
Range (g/d)	0.00-0.36	>0.36-5.26	>5.26-16.00	>16.00-33.89	>33.89-331.70	
Total cases (*n*)	378	218	290	249	203	
RR (95% CI) †	1.0	0.97 (0.81-1.16)	1.02 (0.86-1.20)	0.97 (0.81-1.11)	0.91 (0.75-1.11)	0.39
RR (95% CI) ‡	1.0	1.02 (0.81-1.27)	1.04 (0.85-1.28)	1.05 (0.85-1.30)	0.83 (0.65-1.06)	0.16
RR (95% CI)§	1.0	0.98 (0.73-1.31)	1.12 (0.86-1.46)	0.92 (0.69-1.22)	0.96 (0.71-1.30)	0.61
Pan-fried meat						
Range (g/d)	0.00-1.52	>1.52-4.46	>4.46-9.97	>9.97-23.97	>23.97-424.99	
Total cases (*n*)	283	271	259	276	249	
RR (95% CI) †	1.0	1.02 (0.86-1.20)	0.99 (0.83-1.18)	1.04 (0.88-1.24)	0.94 (0.78-1.13)	0.38
RR (95% CI) ‡	1.0	1.02 (0.84-1.26)	1.02 (0.82-1.25)	0.99 (0.80-1.22)	0.86 (0.68-1.09)	0.09
RR (95% CI)§	1.0	0.93 (0.71-1.20)	0.80 (0.60-1.05)	0.84 (0.63-1.10)	0.86 (0.64-1.15)	0.41
Very well done meat						
Range (g/d)	0	>0-10.0	>10.0	—	—	
Total cases (*n*)	1257	36	45	—	—	
RR (95% CI) †	1.0	1.04 (0.75-1.45)	1.42 (1.05-1.92)	—	—	0.02
RR (95% CI) ‡	1.0	1.19 (0.80-1.76)	1.69 (1.19-2.40)	—	—	0.003
RR (95% CI)§	1.0	1.01 (0.59-1.72)	1.23 (0.73-2.06)	—	—	0.44

NOTE: Models included age, race, study center, family history of prostate cancer, history of diabetes, number of screening exams during follow-up, smoking status, physical activity, aspirin use, body mass index, and intake of total energy, supplemental vitamin E, and lycopene.
Red meat = all beef, pork and lamb (processed and nonprocessed). White meat = poultry and fish. Processed meat = Ham, hot dogs, liver, cold cuts, sausage, bacon.
Very well done meat was considered in three categories, split by the median level of intake in those who consume meat cooked to this degree.
*P_{trend} across quintiles using the median value of each quintile.
†All cases (n = 1,338).
‡Incident cases (n = 868).
§Advanced cases (n = 520).

linear trend used the median value of each quintile. All reported *P*s are two sided. The models were adjusted for race (non-Hispanic White, Black, Asian/Pacific Islander, other), study center (10 indicator variables), family history of prostate cancer (yes/no), body mass index (<25, 25 to <30, ≥30), smoking status (never, current, former, pipe/cigar only), physical activity (hours spent in vigorous activity per week: none, <1, 1, 2, 3, ≥4), total energy intake (kcal/d), supplemental vitamin E use (IU/d: 0, 0-30, >30-400, >400, past use), lycopene intake (μg/d), history of diabetes (yes/no), aspirin use (never, <1/d, ≥1/d), and total number of screening exams during follow-up (as a time-dependent variable).

Results

During follow-up, a total of 1,338 prostate cancer cases were diagnosed, of which 868 were incident cases and 520 were advanced cases. In addition, 9% of the cohort died or were lost to follow-up. The study population was predominately non-Hispanic White (90.7%) followed by Asian/Pacific Islander (4.0%), Black (3.3%), and Hispanic/American Indian/Alaskan (1.9%). Compared with men in the lowest quintile of red meat intake, men in the highest quintile tended to be younger, more likely to be

obese, to have a higher total energy intake, to consume more lycopene, and less likely to use vitamin E supplements (Table 1). High red meat consumers also exercised less, ate less fruits and vegetables, and were more likely to be current smokers (Table 1).

There was no association between red or white meat consumption, meat-cooking method (Table 2), or for meats cooked rare, medium, or well done (data not shown) and risk of total prostate cancer, incident cancer, or advanced disease. The frequency and range of consumption of very well done meat, which contains the highest levels of meat-related mutagens (5, 6), was too small to analyze in quintiles. Considered in three categories, split by the median level of intake (nonconsumers, ≤10 g/d, >10 g/d), there was an elevated risk for total prostate cancer (RR, 1.42; 95% CI, 1.05-1.92; P_{trend} = 0.02) and for incident disease (RR, 1.69; 95% CI, 1.19-2.40; P_{trend} = 0.003), comparing men who consumed >10 g/d to nonconsumers. However, no clear association was evident for advanced disease (RR, 1.23; 95% CI, 0.73-2.06; Table 2).

Processed meat consumption was not associated with overall prostate cancer risk or incident disease (Table 2). The risk of advanced cancer, however, was elevated in those in the highest four quintiles of consumption, although there was no evidence of a dose-response trend (P_{trend} = 0.32).

PhIP intake was associated with an increased risk for overall prostate cancer (highest versus lowest quintile RR, 1.22; 95% CI, 1.01-1.48; P_{trend} = 0.04) and incident disease (highest versus lowest quintile RR, 1.28; 95% CI, 1.01-1.61; P_{trend} = 0.01), although there was no association between PhIP and advanced disease (highest versus lowest quintile RR, 1.06; 95% CI, 0.78-1.43; P_{trend} = 0.59) (Table 3). DiMeIQx, MeIQx, and BaP intake or overall mutagenic activity from meat was not associated with prostate cancer risk. Correlations show that PhIP was weakly correlated with red meat (r = 0.15, P < 0.001) and very well done meat consumption (r = 0.16, P = <0.001) and had slightly higher correlations with MeIQx (r = 0.22, P < 0.001) and DiMeIQx (r = 0.37, P < 0.001).

Table 3. Distribution and RRs with 95% CIs for prostate cancer risk according to quintiles of HCAs and BaP

Variable	Quintile					P_{trend}*
	1 (reference)	2	3	4	5	
DiMeiQx						
Range (ng/d)	0.0-0.2	>0.2-0.7	>0.7-1.6	>1.6-3.4	>3.4-159.0	
Total cases (n)	277	310	276	242	233	
RR (95% CI) †	1.0	1.14 (0.96-1.35)	1.04 (0.86-1.25)	0.94 (0.76-1.16)	0.98 (0.77-1.25)	0.40
RR (95% CI) ‡	1.0	1.09 (0.89-1.34)	1.11 (0.89-1.41)	0.93 (0.71-1.22)	0.98 (0.71-1.33)	0.37
RR (95% CI)§	1.0	0.93 (0.71-1.23)	1.00 (0.74-1.36)	1.04 (0.74-1.46)	1.03 (0.69-1.53)	0.75
MeIQx						
Range (ng/d)	0.0-9.8	>9.8-19.4	>19.4-33.1	>33.1-59.4	>59.4-1230.8	
Total cases (n)	299	300	260	242	237	
RR (95% CI) †	1.0	1.04 (0.88-1.24)	1.00 (0.82-1.22)	0.97 (0.78-1.21)	0.97 (0.76-1.24)	0.67
RR (95% CI) ‡	1.0	1.03 (0.83-1.26)	0.84 (0.66-1.08)	0.87 (0.67-1.15)	0.90 (0.66-1.22)	0.49
RR (95% CI)§	1.0	1.08 (0.82-1.43)	0.94 (0.68-1.31)	0.99 (0.70-1.42)	0.95 (0.64-1.43)	0.61
PhIP						
Range (ng/d)	0.0-25.5	>25.5-56.1	>56.1-112.7	>112.7-269.2	>269.2-7862.9	
Total cases (n)	280	280	272	247	259	
RR (95% CI) †	1.0	1.05 (0.88-1.24)	1.07 (0.89-1.27)	1.04 (0.87-1.25)	1.22 (1.01-1.48)	0.04
RR (95% CI) ‡	1.0	0.98 (0.80-1.22)	1.07 (0.86-1.33)	1.00 (0.79-1.26)	1.28 (1.01-1.61)	0.01
RR (95% CI)§	1.0	1.00 (0.76-1.30)	1.01 (0.76-1.33)	0.88 (0.65-1.18)	1.06 (0.78-1.43)	0.59
BaP						
Range (ng/d)	0.0-1.2	>1.2-4.2	>4.2-18.0	>18.0-64.6	>64.6-1031.5	
Total cases (n)	305	282	287	244	220	
RR (95% CI) †	1.0	1.00 (0.85-1.18)	1.01 (0.86-1.19)	0.93 (0.78-1.10)	0.94 (0.79-1.13)	0.36
RR (95% CI) ‡	1.0	0.96 (0.78-1.18)	1.07 (0.87-1.30)	0.94 (0.76-1.16)	0.95 (0.76-1.18)	0.54
RR (95% CI)§	1.00	0.85 (0.65-1.11)	0.95 (0.74-1.23)	0.94 (0.72-1.22)	0.85 (0.64-1.13)	0.49
Mutagenic activity (per 1,000 revertant colonies/d)						
Range (μg/d)	0.0-1.7	>1.7-3.4	>3.4-5.9	>5.9-11.4	>11.4-503.0	
Total cases (n)	301	294	264	240	239	
RR (95% CI) †	1.0	1.02 (0.87-1.21)	1.01 (0.85-1.19)	0.98 (0.82-1.16)	1.04 (0.87-1.24)	0.73
RR (95% CI) ‡	1.0	0.98 (0.81-1.19)	0.87 (0.71-1.07)	0.86 (0.69-1.07)	1.03 (0.83-1.28)	0.56
RR (95% CI)§	1.0	0.85 (0.65-1.11)	0.96 (0.74-1.26)	1.03 (0.79-1.36)	0.94 (0.71-1.26)	0.97

NOTE: Multivariate models included age, race, study center, family history of prostate cancer, history diabetes, number of screening exams during follow-up, smoking status, physical activity, aspirin use, body mass index, and intake of total energy, supplemental vitamin E, and lycopene.

*P_{trend} across quintiles using the median value of each quintile.

†All cases (n = 1,338).

‡Incident cases (n = 868).

§Advanced cases (n = 520).

Discussion

In this cohort study, consumption of >10 g/d of very well done meat was associated with a 42% increased risk for prostate cancer and a 69% increased risk for incident disease. Furthermore, a high PhIP intake was associated with a 22% increased risk for prostate cancer and a 28% increased risk for incident disease.

Previous cohort studies have yielded conflicting results with regard to red meat and prostate cancer risk, with some studies suggesting a positive association (21–24), whereas others have found no association (25, 26). The results from this study do not support the hypothesis that red meat intake per se is a risk factor for prostate cancer.

The one previous epidemiologic study of HCAs and prostate cancer, a population-based case-control study of 317 cases in New Zealand, found that individuals who consumed well-done beef steak had a 1.7-fold (95% CI, 1.02-2.77; P_{trend} = 0.03) increased risk of prostate cancer compared with those who did not consume well-done beef steak. This case-control study did not, however, find an association between prostate cancer and HCAs, including PhIP (odds ratio, 1.05; 95% CI, 0.70-1.59); nevertheless, consideration must be given to the study's size and the retrospective design (14).

The mean intakes of the three HCAs and BaP investigated in our study were similar to those reported previously from western populations (27–29). Although HCAs are formed in the highest concentrations in very well done meats cooked by high-temperature cooking methods, the correlation between PhIP and very well done meat was not particularly high (r = 0.16). The frequency of consumption of very well done meats is generally low; therefore, the majority of PhIP intake derives from less well done meats that are more frequently consumed. In our study population, the main source of PhIP was barbecued chicken (54%), with smaller contributions from broiled and fried chicken (17%), steak cooked to a medium level of doneness (26%) and well-done hamburgers (2%).

The lack of association with DiMeIQx, MeIQx, and BaP is in agreement with much of the animal literature, which also finds risks specifically associated with PhIP (7, 8). There are several lines of evidence to support the notion that PhIP may increase prostate cancer risk. In contrast to the majority of HCAs, PhIP targets the prostate tissues (30). Furthermore, PhIP is the most abundant HCA, one of the most readily absorbed mutagenic HCAs found in cooked meat (31) and has the highest carcinogenic potential of the HCAs (32). In rodents, PhIP increases mutation frequency in prostate tissue (7) and when administered in the diet, yields a high number of rodent prostate cancers (8).

Studies conducted with human tissue complement the animal data. Human prostate tissue is known to express CYP and NAT enzymes (10–12), which metabolically activate HCAs, leading to increased DNA adduct levels in response to PhIP (33). In addition, treatment of primary cell cultures from human prostate tissue with PhIP results in dose-dependent genotoxic damage (34, 35).

The mechanism of PhIP induced prostate cancer is uncertain. In addition to its direct mutagenic activity, animal experiments have identified similarities between PhIP and estradiol, both of which have been associated with cancers of the prostate (30, 36). In rodents, PhIP exhibits estrogenic activity, potentially through sex steroid receptor binding, whereas other HCAs, such as MeIQx, do not (37, 38). Furthermore, PhIP, at levels equivalent to those encountered in the human diet, and estradiol both result in increased cell proliferation *in vitro* and have other similar mitogenic responses (38).

The strengths of this study include the detailed information on screening procedures that could be used to control for screening frequency (39) and also the detailed information on meat and meat-cooking practices, which enabled the determination of HCA and BaP intake. Consideration must be given, however, to the measurement error and associated attenuation of risks inherent to dietary studies based on questionnaire data. Although the FFQ had very specific and detailed questions on meat intake and meat-cooking practices, derived from a validated questionnaire (29), we did not consider marinating of meat or flipping of hamburgers, both of which can affect the formation of HCAs and BaP (40, 41). Although we considered a large number of potential confounders, prostate cancer is a disease with few known risk factors; therefore, we cannot be sure that we identified all confounding variables.

In conclusion, the results of this prospective study found that a high intake of very well done meat and a high intake of PhIP were both positively associated with prostate cancer risk, lending epidemiologic support to experimental observations. If confirmed in further studies, PhIP would be the first chemical carcinogen associated with prostate cancer in human studies.

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